

**ANTIBACTERIAL ACTIVITY OF *ACMELLA PANICULATA*
EXTRACTS AGAINST ORAL BACTERIA (*STREPTOCOCCUS*
MUTANS)**

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A thesis submitted in partial fulfilment for the degree of
DOCTOR OF PHILOSOPHY IN
SCIENCE AND TECHNOLOGY

UNIVERSITI SAINS ISLAM MALAYSIA

July 2023

AUTHOR DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

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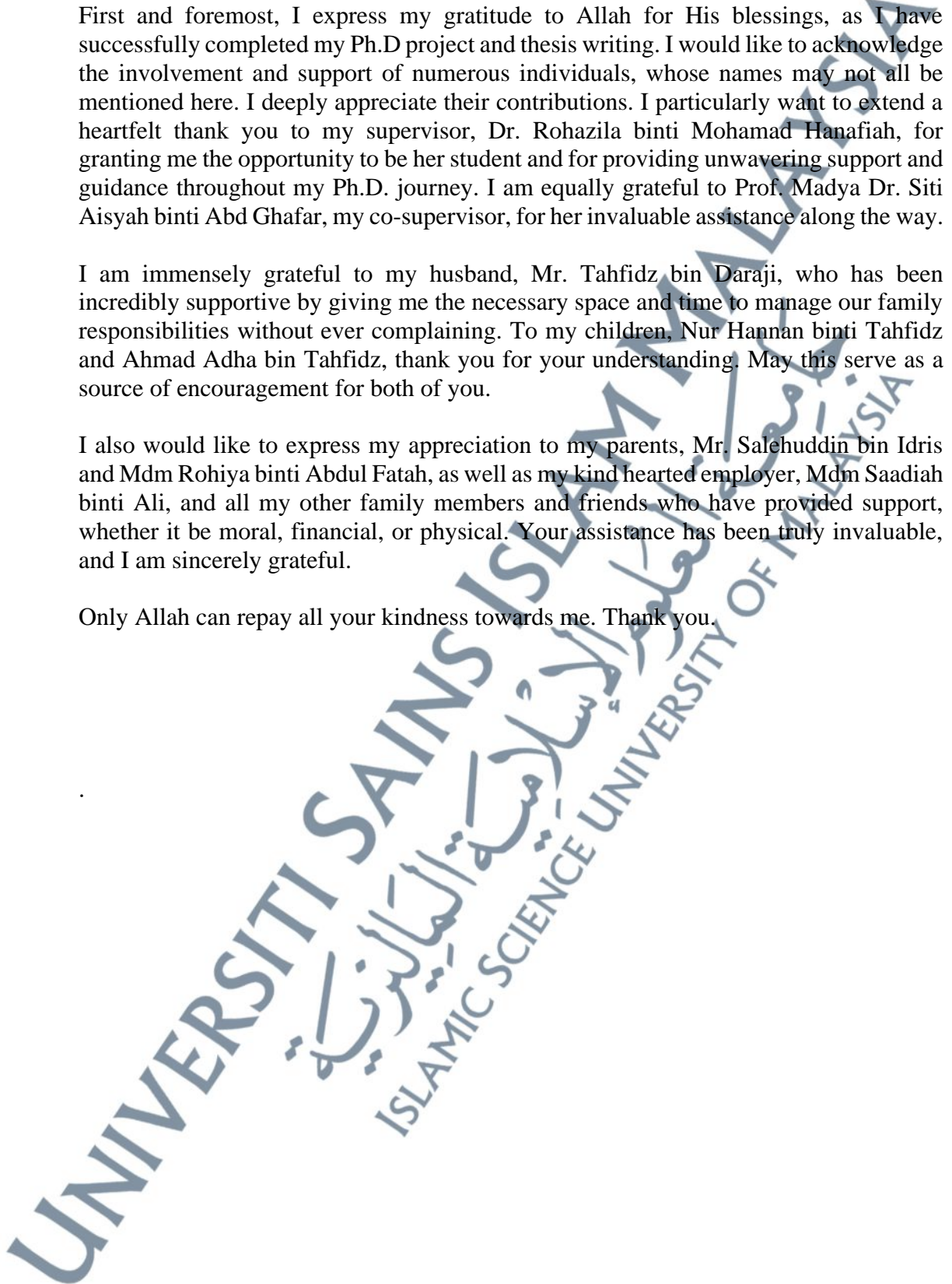
ACKNOWLEDGEMENT

First and foremost, I express my gratitude to Allah for His blessings, as I have successfully completed my Ph.D project and thesis writing. I would like to acknowledge the involvement and support of numerous individuals, whose names may not all be mentioned here. I deeply appreciate their contributions. I particularly want to extend a heartfelt thank you to my supervisor, Dr. Rohazila binti Mohamad Hanafiah, for granting me the opportunity to be her student and for providing unwavering support and guidance throughout my Ph.D. journey. I am equally grateful to Prof. Madya Dr. Siti Aisyah binti Abd Ghafar, my co-supervisor, for her invaluable assistance along the way.

I am immensely grateful to my husband, Mr. Tahfidz bin Daraji, who has been incredibly supportive by giving me the necessary space and time to manage our family responsibilities without ever complaining. To my children, Nur Hannan binti Tahfidz and Ahmad Adha bin Tahfidz, thank you for your understanding. May this serve as a source of encouragement for both of you.

I also would like to express my appreciation to my parents, Mr. Salehuddin bin Idris and Mdm Rohiya binti Abdul Fatah, as well as my kind hearted employer, Mdm Saadiah binti Ali, and all my other family members and friends who have provided support, whether it be moral, financial, or physical. Your assistance has been truly invaluable, and I am sincerely grateful.

Only Allah can repay all your kindness towards me. Thank you.



ABSTRAK

Acmella paniculata, yang dikenali sebagai tumbuhan sakit gigi, telah digunakan secara meluas sebagai ubat tradisional untuk membantu merawat penyakit yang berkaitan dengan kesakitan gigi dan jangkitan gusi. *Streptococcus mutans* merupakan bakteria utama yang menyebabkan berlakunya masalah karies gigi. Kajian ini bertujuan untuk mengenalpasti aktiviti antibakteria ekstrak *A. paniculata* terhadap *S. mutans*. Lapan sampel daun dan bunga *A. paniculata* telah berjaya diekstrak menggunakan *n*-heksana, diklorometana, aseton dan metanol. Kesemua sampel diuji untuk aktiviti antibakteria menggunakan ujian resapan cakera, ujian perencatan minimum (MIC) dan ujian bakterisidal minimum (MBC). Hasil saringan mendapati, hanya ekstrak daun *n*-heksana (APLHE), ekstrak daun metanol (APLME), ekstrak bunga *n*-heksana (APFHE), dan ekstrak bunga diklorometana (APFDE) menunjukkan kesan aktiviti antibakteria terhadap *S. mutans*. Oleh itu, keempat-empat sampel ini kemudiannya diuji dengan ujian antibiolapisan dan ujian kadar masa membunuh terhadap *S. mutans*. Keputusan menunjukkan bahawa ekstrak bunga mempunyai lebih banyak aktiviti antibakteria terhadap *S. mutans* berbanding dengan ekstrak daun. Oleh itu, mikroskop elektron pengimbas (SEM) dan mikroskop elektron transmisi (TEM) telah digunakan untuk melihat keadaan morfologi *S. mutans* di bawah rawatan bersama APFHE dan APFDE. Melalui kedua-dua pemerhatian mikrograf, bentuk *S. mutans* berubah dari kokus dan berbulat kepada memanjang dan lisis selepas rawatan. Kemudian, dengan menggunakan GC-MS, sebatian fitokimia utama yang ditemui dalam kedua-dua APFHE dan APFDE adalah asid lemak (seperti asid heksadekanoik, asid oleik, dan asid linoleik). Akhir sekali, analisis transkriptomik dilakukan terhadap *S. mutans* yang telah dirawat dengan APFDE. Keputusan membuktikan bahawa APFDE mempengaruhi biosintesis peptidoglikan, ekspresi gen, penderiaan kuorum, kitaran sitrat dan laluan glikolisis *S. mutans*. Kesimpulannya, aktiviti antibakteria APFDE telah mengganggu pelbagai proses biologi dan laluan *S. mutans*.

Kata kunci: Aktiviti antibakteria, *Acmella paniculata*, *Streptococcus mutans*

ABSTRACT

Acmella paniculata, popularly known as the toothache plant, has been widely used as a traditional medicine to help treat diseases associated with toothache and gum infections. *Streptococcus mutans* is a common bacterium that can cause dental caries. This study aims to determine the antibacterial activity of *A. paniculata* extracts against *S. mutans*. Eight samples of *A. paniculata* leaves and flowers had been successfully extracted using *n*-hexane, dichloromethane, acetone and methanol. All samples were tested for antibacterial activity using the disc diffusion assay, the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC). From the screening, only *n*-hexane leaves extract (APLHE), methanol leaves extract (APLME), *n*-hexane flower extract (APFHE) and dichloromethane flower extract (APFDE) exhibited antibacterial activity against *S. mutans*. Therefore, all four samples were tested with an antibiofilm and time-kill assay to determine the reduction activity and time-kill rate against *S. mutans*. The results showed that flower extracts exhibit more antibacterial activity against *S. mutans* when compared with leaves extracts. Thus, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to examine the morphology of *S. mutans* under treatment with APFHE and APFDE. According to both micrograph observations, the shape of *S. mutans* changed from coccus and rounded to elongated and lysed after treatments. Then, by using GC-MS, the major phytochemical compound found in both APFHE and APFDE is fatty acids (such as hexadecanoic acid, oleic acid, and linoleic acid). Finally, transcriptomic analysis was performed on *S. mutans* that had been treated with APFDE. The results proved that APFDE affected the biosynthesis of peptidoglycan, gene expression, quorum sensing, citrate cycle and glycolysis pathway of *S. mutans*. In conclusion, the antibacterial activity of APFDE disrupted various biological processes and pathways of *S. mutans*.

Keywords: Antibacterial activity, *Acmella paniculata*, *Streptococcus mutans*

الملخص

تم استخدام أكميلة / البابونج *Acmella paniculata* أو المعروفه باسم نبات لعلاج ألم الأسنان على نطاق واسع كدواء تقليدي للمساعدة في علاج الأمراض المرتبطة بألم الأسنان والتهابات اللثة. العقديّة الطافرة *Streptococcus mutans* هي البكتيريا الشائعة التي يمكن أن تسبب تسوس الأسنان. تهدف هذه الدراسة إلى تحديد النشاط المضاد للبكتيريا لمستخلصات *A. paniculata* ضد *S. mutans*. تم استخراج ثمانية عينات من أوراق وأزهار أكميلة / البابونج *A. paniculata* بنجاح باستخدام الهكسان العادي و كلوريد الميثيلين والأسيتون و الميثانول. من أجل التحقيق في النشاط المضاد للبكتيريا، خضعت جميع العينات لمقاييس انتشار القرص، وتحديد الحد الأدنى للتركيز المثبط والحد الأدنى لتركيز مبيد الجراثيم (MBC\MIC). من الفحص، أظهرت فقط مستخلص أوراق الهكسان العادي (APLHE)، ومستخلص أوراق الميثانول (APLME)، ومستخلص زهرة الهكسان العادي (APFHE) ومستخلص زهرة كلوريد الميثيلين (APFDE) نشاطاً مضاداً للبكتيريا ضد *S. mutans*. لذلك، تم اختبار جميع العينات الأربعة باستخدام مكافحة الغشاء الحيوي ومقاييس وقت القتل لتحديد نشاط الاختزال ومعدل وقت القتل ضد *S. mutans*. أظهرت النتائج أن مستخلصات الأزهار تظهر فعالية أكبر كمضاد للبكتيريا ضد *S. mutans* بالمقارنة مع مستخلصات الأوراق. وبالتالي، تم إجراء تحليل التشكل تجاه *S. mutans* باستخدام APFDE و APFHE باستخدام المجهر الإلكتروني الماسح (SEM) المجهر الإلكتروني النافذ (TEM). وفقاً لكل من الملاحظة المجهرية، تغير شكل خلايا *S. mutans* من كروية ومدوّرة إلى خلايا ممدودة الشكل وتحلل بعد المعالجة. بعد ذلك، باستخدام مقياس الطيف الكتلي اللوني للغاز GC-MS، يكون المركب الكيميائي النباتي الرئيسي الموجود في كل من APFDE و APFHE هو الأحماض الدهنية (مثل حمض الهكساديكانويك، حمض الأوليك، وحمض اللينوليك). تم إجراء التحليل الترانسكريبي على *S. mutans* الذي تم علاجه ب APFDE. أثبتت النتائج أن APFDE تؤثر على تخليق الببتيدوجليكان وتعبير الجينات والاستشعار الغوغائي ودورة السترات ومسار الجلوكوليز لـ *S. mutans*. وباختصار، فإن النشاط المضاد للجراثيم لـ APFDE يعمل على تعطيل العديد من العمليات الحيوية والمسارات في *S. mutans*.

الكلمات الدالة: النشاط المضاد للبكتيريا، أكميلة / البابونج، العقديّة الطافرة (*Streptococcus mutans*).

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LIST OF ABBREVIATIONS

AEP	Acquired Enamel Pellicle
APF	<i>A. paniculata</i> flower
APFDE	<i>A. paniculata</i> flower Dichloromethane extract
APFHE	<i>A. paniculata</i> flower <i>n</i> -hexane extract
APL	<i>A. paniculata</i> leaves
APLHE	<i>A. paniculata</i> leaves <i>n</i> -hexane extract
APLME	<i>A. paniculata</i> leaves methanol extract
ATCC	American Type Culture Collection
BHI	Brain Heart Infusion
BHIB	Brain Heart Infusion Broth
Ca	Calcium
CFU	Colony Forming Unit
COX	Cyclooxygenases
DCM	Dichloromethane
DEG	Database of Essential Genes
DEG	Differential of Sequencing Gene
Dex	Dextranase
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
EPS	Extracellular Polysaccharide
FPKM	Fragments Per Kilobase of Transcript Sequence per Millions Base Pair-Sequenced
FruA	Fructanase
Ftf	Fructosyltransferase
GBPs	Glucan Binding Protein
GC-MS	Gas Chromatography-Mass Spectrometry
GO	Gene Ontology
<i>gtf</i>	Glycosyltransferase
HPLC	High-Performance Liquid Chromatography
IBA	Indolebutyric Acid
IL	Interleukin
iNOS	Inducible Nitric Oxide Synthase
IPS	Intercellular Polysaccharide
KEGG	Kyoto Encyclopedia of Genes and Genomes
LDH	Lactate Dehydrogenase
MAPK	Mitogen-Activated Protein Kinase
MBA	Methylbutylamide
MBC	Minimum Bactericidal Concentration
MF	Molecular Function
MHA	Muller Hinton agar
MHB	Muller Hinton broth
MIC	Minimum Inhibitory Concentration
NF	Nuclear Factor
NMR	Nuclear Magnetic Resonance
NO	Nitric oxide

PCR	Polymerase Chain Reaction
PDMS	Polydimethylsiloxane
PO	Phosphates
QC	Quality Control
RNA	Ribonucleic Acid
SEM	Scanning Electron Microscope
SM	<i>Streptococcus mutans</i>
SpaP	Surface-associated Protein
SPME	Solid Phase Microextraction
TE	Tris-EDTA
TEM	Transmission Electron Microscope

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